Court A3

142-163, American Chemical Society, Washington. See also Tomme, P. & Claeyssens, M. (1989) FEBS Lett. 243, 239-243l; Gilkes, N.R et al., (1988) J.Biol.Chem. 263, 10401-10407.

REMARKS

The present paper is submitted as a complete response to the Notice mailed August 22, 2001. Applicants respectfully request that the present papers be made of record.

Should any additional issues need to be resolved, the Examiner is requested to telephone the undersigned to attempt to resolve those issues. If a further written action is required, Applicant requests that the prior final rejection be withdrawn for the reasons noted above.

Respectfully submitted,

Paul J. White, Reg. No. 30,436
Attorney for Applicants

Dated: October 22, 2001.

National Renewable Energy Laboratory
1617 Cole Blvd.
Golden, CO 80401
303/384-7575

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: William S. Adney, et al. Docket No.: NREL 01-38

Serial No.: 09/917,384

Filed: July 28, 2001

Title: THERMAL TOLERANT ~~EXO~~GLUCANASE FROM ACIDOTHERMUS CELLULOLYTICUS

AMENDMENTS -- MARKUP

Please delete the second full paragraph on page 3, beginning line 21.

[The potential exists for the successful, commercial-scale expression of heterologous cellulases, and in particular novel cellulases with or without any one or more desirable properties such as thermal tolerance and resistance to acid inactivation, proteolytic inactivation, and solvent inactivation. Such expression can occur in filamentous fungi, bacteria, and other hosts.]

Please replace the second full paragraph on page 13, beginning line 14, with the following:

"Thermal tolerant" refers to the property of withstanding partial or complete inactivation by heat and can also be described as thermal resistance or thermal stability. Although some variation exists in the literature, the following definitions can be considered typical for the optimum temperature range of stability and activity for enzymes: psycrhophilic (below freezing to 10C); mesophilic (10°C to 50°C); thermophilic (50°C to 75°C); and caldophilic (75°C to above boiling water temperature). The stability and catalytic activity of enzymes are linked characteristics, and the ways of measuring these properties vary considerably. For industrial enzymes, stability and activity are best measured under use conditions, often in the presence of substrate. Therefore, cellulases that must act on process streams of cellulose must be able to withstand exposure up to thermophilic or even caldophilic temperatures for digestion times in excess of several hours.

Please replace the second full paragraph on page 15, beginning line 16, with the following:

Cellulases belong to the GH family of enzymes. Cellulases are produced by a variety of bacteria and fungi to degrade the beta-(1,4)-[β-1,4]glycosidic bond of cellulose and to so produce successively smaller fragments of cellulose and ultimately produce glucose. At present, cellulases are found in[within are] at least 11 different GH families. Three

different types of cellulase enzyme activities have been identified within these GH families: exo-acting cellulases which cleave successive disaccharide units from the non-reducing ends of a cellulose chain; endo-acting cellulases which randomly cleave successive disaccharide units within the cellulose chain; and β-glucosidases which cleave successive disaccharide units to glucose (J. W. Deacon, (1997) Modern Mycology, 3rd Ed., ISBN: 0-632-03077-1, 97-98).

Please replace the first full paragraph on page 17, beginning line 4, with the following:

Gux1, as noted above, has a catalytic domain, identified as belonging to the GH48 family. The GH48 domain family includes a number of exoglucanases, for example, from *Cellulomonas fimi*, and exoglucanase E6[E3] isolated from *Thermobifida fusca*. The GH48 members degrade substrate using an inverting mechanism. Being a member of the GH48 family of proteins identifies Gux1 as potentially having exoglucanase activity. In addition, the predicted amino acid sequence (SEQ ID NO: 1) indicates that CBD type II and CBD type III domains are present as characterized by Tomme P. et al. (1995), in Enzymatic Degradation of Insoluble Polysaccharides (Saddler JN & Penner M, eds.), at 142-163, American Chemical Society, Washington. See also Tomme, P. & Claeyssens, M. (1989) FEBS Lett. 243, 239-2431; Gilkes, N.R et al., (1988) J.Biol.Chem. 263, 10401-10407.